AN X-RAY SCATTERING STUDY OF BROMEGRASS MOSAIC VIRUS

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ABSTRACTS X-ray scattering data and electron microscope observations are presented for bromegrass mosaic virus. Its radial density distribution is obtained from the Fourier transform of the amplitudes of the scattered x-rays. The results indicate that the virus is 260 A in diameter, it has an almost empty central cavity which is about 80 A in diameter, and the regions occupied by RNA and protein are approximately equal in average density. Electron micrographs of negatively stained preparations also give an outside diameter of 260 A and indicate that there is a central region about 90 A in diameter into which uranyl acetate can penetrate. Positively stained preparations indicate that the nucleic acid is concentrated in a shell-shaped region which is in turn surrounded by a shell of protein. In order for the RNA and protein regions to have the same average electron density the RNA must have a hydration of 1.29 gm of water per gm of RNA and the protein must have a hydration of 0.24 gm of water per gm of protein.

INTRODUCTION

X-ray scattering experiments on solutions of a number of small "spherical" viruses have been carried out in this laboratory. The aim has been to obtain information about the structure of these viruses, particularly those features which are common to all of them. Previous papers have reported on southern bean mosaic virus, tobacco necrosis virus, and tomato bushy stunt virus (Leonard, Anderegg, Shulman, Kaesberg and Beeman, 1953), top and bottom components of turnip yellow mosaic virus (Schmidt, Kaesberg, and Beeman, 1954) and top and bottom components of wild cucumber mosaic virus (Anderegg, Geil, Beeman, and Kaesberg, 1961). In this paper we report a similar x-ray study of bromegrass mosaic virus (BMV) and correlate this information with electron micrographs of BMV stained with uranyl acetate.

BMV is a plant virus with a particularly low molecular weight and nucleic acid content. Bockstahler and Kaesberg (1962) have measured its molecular weight to be 4.6×10^6 and its RNA content to be 21.4 per cent. Thus the molecular weight of the viral RNA is 1.0×10^6. Electron micrographs of frozen-dried, shadowed

preparations of BMV show particles having more or less hexagonal contours with the distance between the vertices about 290 A (Kaesberg, 1959). The present results indicate that the virus in solution is roughly spherical with a diameter of 260 A and that it consists of a shell of nucleic acid surrounded by a shell of protein. The nucleic acid does not extend all the way to the center thus leaving a central cavity approximately 80 A in diameter.

EXPERIMENTAL

The x-ray source was a rotating copper anode tube operating at 30 kv and 80 ma. The slits which were used to collimate the x-ray beam incident on the sample and to analyze the scattered beam were 1 cm high and were separated by 50 cm. Slit widths were 0.015 cm for the radius of gyration determinations and 0.045 cm for the data covering a larger angular range. Monochromatization of the x-rays was accomplished by using a proportional counter and a single-channel pulse-height analyzer. A nickel filter reduced the intensity of Cu K_β radiation to about 3 per cent of Cu K_α. Smearing effects due to the height and width of the collimating apertures were corrected for with the help of an IBM 650 computer. The background to be subtracted from each scattering curve was determined by making a run with only solvent in the sample holder.

Purification of the virus is described by Bockstahler and Kaesberg (1962). After the final centrifugation the virus was suspended in 0.15 M NaCl at a pH of approximately 6.0 and these solutions were diluted appropriately for the x-ray work.

Samples were prepared for electron microscopy using standard staining techniques with uranyl acetate (Brenner and Horne, 1959; Huxley and Zubay, 1960*a*; Huxley and Zubay, 1961). A drop of BMV solution at a concentration of 0.01 per cent was placed on a collodion film on a specimen grid. For positive staining a drop of uranyl acetate solution at a concentration of 0.5 per cent was added. After 10 minutes the solution remaining on the grid was absorbed with filter paper; the sample was dried for 1 hour at room temperature, and finally washed with a droplet of distilled water. For negative staining the concentration of the uranyl acetate solution was 2 per cent and the final washing was omitted.

RESULTS

Radius of Gyration. The apparent radius of gyration at several concentrations was calculated from the slope of the straight line obtained by plotting the log of the scattered intensity *versus* the square of the scattering angle (Guinier and Fournet, 1955, p. 126). The results obtained after making a small correction due to the finite dimensions of the slits were 108 A, 105 A and 110 A at concentrations of 1.0, 0.5, and 0.25 per cent, respectively. The variation with concentration was less than the experimental precision so an average value of 108 A was chosen.

Radial Density Distribution. A solution of 16 per cent BMV was used to determine the shape of the scattering curve over a large angular range. Interparticle interference effects were eliminated by combining these data with those

taken at a concentration of 1.0 per cent. The scattering curve after smoothing and correction for the finite dimensions of the slits is shown in Fig. 1.

The scattering curve shows a strong central maximum and six subsidiary maxima. The maxima agree quite well in position with those in the scattering curve for a sphere of uniform density 260 A in diameter. The intensity of the maxima, however, do not agree. Assuming that the virus is approximately spherically symmetric, the radial distribution of electron density can be determined from the

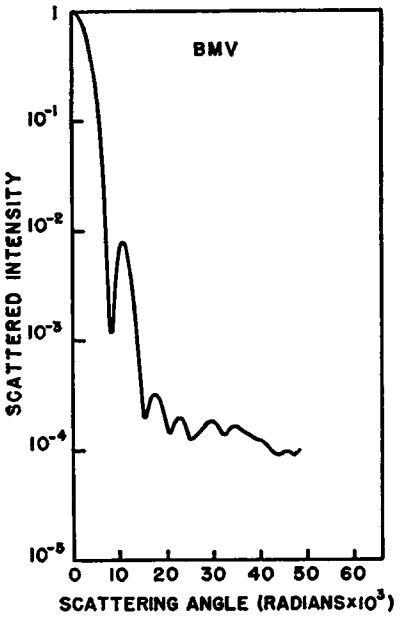

FIGURE 1 The intensity of x-rays scattered by a solution of bromegrass mosaic virus as a function of scattering angle. The data have been smoothed and corrected for the effects of size of the collimating slits. The intensities are on a log plot because of the large range.

Fourier transform of the scattered amplitudes (Guinier and Fournet, 1955, p. 28). We made this calculation assuming that the amplitudes in the central maximum were positive and that the amplitudes for succeeding maxima alternate in sign, as they do for uniform spheres and for spheres with a hollow center. Only the first five subsidiary maxima were used in calculating the transform. Spurious diffraction effects due to the cut-off of the data were reduced by multiplying by an artificial temperature factor consisting of a Gaussian function with the constant chosen so as to multiply the data by 0.1 at the cut-off point. The calculated transform is shown in Fig. 2*a*.

It may be noticed, first of all, from the transform that the virus is hollow. Due to the limited resolution in the transform it is difficult to say whether the hollow region is completely devoid of protein and nucleic acid. Assuming that there is a central region of the virus occupied only by water and that the boundary of this region as well as the outer surface of the virus are sharp but have been smeared

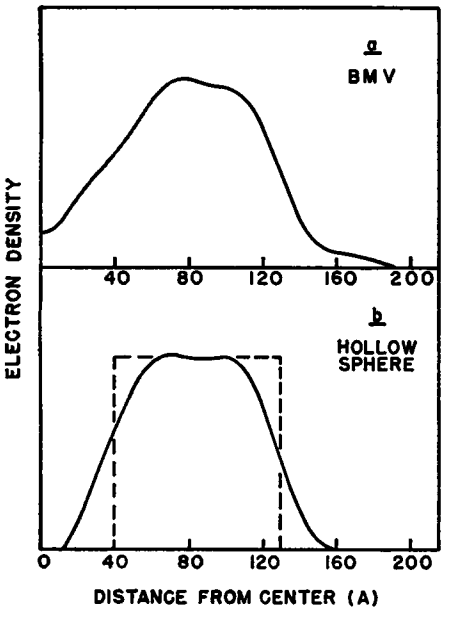

FIGURE 2*a* The radial electron density distribution inside bromegrass mosaic virus as calculated from the Fourier transform of the amplitudes of the scattered x-rays.

FIGURE 2*b* The Fourier transform (solid curve) of the theoretically calculated scattering amplitudes for a hollow sphere of inner radius 40 A and outer radius 130 A. In order to simulate the resolution in the transform for BMV we have cut off the scattering curve at the same point and used the same artificial temperature factor. The dashed line is the transform which would result if the entire amplitude curve had been used in the calculation.

out symmetrically in calculating the transform, one would estimate that the boundary of the inner core is at a radius of 40 A and the outer surface of the virus is at a radius of 130 A. Accordingly, we have calculated the scattering function for a 260 A sphere with an 80 A hollow core and then calculated the Fourier transform of these scattering amplitudes making use of only the first five subsidiary maxima, so as to simulate the resolution in our transform for BMV. The result is shown in Fig. 2*b*. There is considerable agreement between the theoretical transform and the actual transform for BMV. The latter, however, does not go down to the density of water at the origin as does the theoretical transform. Little significance can be attached to this since the shape of the transform near the origin is very sensitive to the point at which the scattering curve is cut off.

Electron Micrographs. A typical micrograph of negatively stained BMV particles is shown in Fig. 3*a*. The particles are outlined by the uranyl acetate in which they are embedded. They appear quite regular in size and approximately circular in projection with a diameter of about 260 A. Some of the particles show a vaguely hexagonal cross-section. All of the particles in this picture show a dark region in the center which is about 90 A in diameter. Apparently this is due to uranyl acetate which has penetrated into the hollow center of the virus. Occasional particles (not shown) have a much larger dark region in the center; presumably they are particles lacking RNA. The outer edge of the virus particles appears knobby but the knobs are too indistinct to count.

A typical example of a positively stained preparation is shown in Fig. 3*b*. The

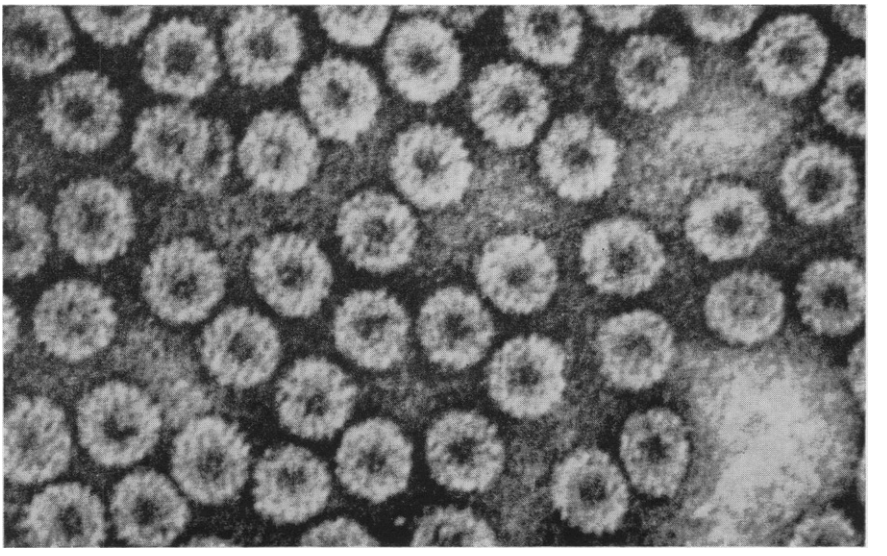

FIGURE 3*a*

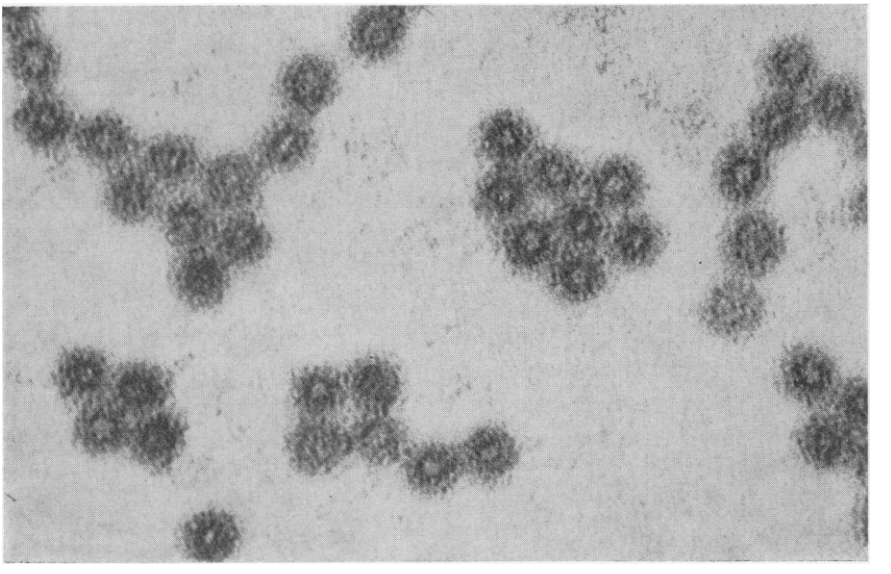

FIGURE 3*b*

FIGURE 3 Electron micrographs of bromegrass mosaic virus stained with uranyl acetate. The magnification is 420,000 ×. (*a*) Negative staining, (*b*) positive staining.

virus particles now appear dark against a light background apparently because the virus has taken up the stain while the background is free of it. The particles are much less distinct than in the negatively stained preparation, but in many cases one sees a lightly stained center surrounded by a ring of more densely stained material which is in turn surrounded by another lightly stained ring. There is evidence (Huxley and Zubay, 1961) that uranyl acetate stains nucleic acid preferentially, but that it also stains protein. The micrographs of positively stained particles thus appear to be consistent with a model of the virus consisting of a hollow center surrounded by a shell of nucleic acid which in turn is covered by a protein coat. The images are not clear enough for accurate measurements but the over-all diameter of the particles is about 240 A.

Hydration. The transform for BMV indicates that the region occupied by RNA has an average electron density nearly the same as that of the region occupied by protein. Since the partial specific volume of RNA is much less than that of protein, this uniformity of electron density indicates that there is more water associated with the RNA than with the protein. We shall call this water of hydration although it should be made clear that we have no evidence that any of this water is bound rather than free water. Assuming that the regions occupied by RNA and protein do not overlap, that they have the same average electron density, and that all water of hydration has normal density, then from the virus dimensions given above and from the known molecular weights and partial specific volumes of the RNA and protein components of BMV one can calculate the hydration of the RNA and protein. Bockstahler and Kaesberg (1962) give the partial specific volume of BMV virus, protein, and RNA as 0.708, 0.751, and 0.550, respectively. Thus the product of the molecular weight of the virus times its partial specific volume gives a volume of 5.40×10^6 A^3. The volume of 260 A sphere is 9.20×10^6 A^3. The difference must be the volume of the water of hydration if we assume normal density for the water. This amounts to a hydration of 0.50 gm of water per gm of virus. The water to fill an 80 A sphere in the center of the virus would amount to 0.03 gm of water per gm of virus. If the remaining water is divided so as to equalize the average electron density of the RNA and protein regions we calculate that there must be 0.24 gm of water per gm of protein and 1.29 gm of water per gm of RNA. The electron densities of both regions would then be 1.21 times that of water; the hydrated RNA would occupy the region between 40 A and 95 A and the hydrated protein would occupy the 35 A shell from a radius of 95 A out to 130 A.

DISCUSSION

The most interesting result of this investigation is that bromegrass mosaic virus has a hollow center. Tobacco mosaic virus is also known to have a hole down the center (Caspar, 1956) but this is a cylindrical molecule and the structure is en-

tirely different than in the present case. There was some suggestion of a small hollow in wild cucumber mosaic virus (Anderegg *et al.*, 1961) but the resoultion was not good enough to be sure. With BMV both the x-ray data and the negatively stained electron micrographs indicate that there is a hollow central cavity 80 to 90 A in diameter. We cannot be certain that this region is entirely devoid of RNA but at least the density is much lower than in the rest of the virus. The radial density distribution curve indicates that perhaps the density drops off gradually inside a radius of about 70 A. Presumably repulsive forces between the different parts of the RNA chains have forced the RNA away from the center and out against the protein coat which surrounds it.

The transform of the x-ray data and the electron micrographs of negatively stained preparations both give an outer diameter for BMV of 260 A, while the positively stained particles appear about 240 A in diameter. Thus the negatively stained preparations give no evidence for shrinkage of the virus during drying. This is contrary to the results of Brenner and Horne (1959) and Huxley and Zubay (1960*b*) on turnip yellow mosaic virus. They found the diameter of the negatively stained virus to be considerably smaller than the diameter of the wet particle as determined from x-ray data. That there is a real difference in these two cases is perhaps borne out by the fact that the pictures of turnip yellow mosaic virus show no particles that touch one another, while our pictures of BMV show many particles in apparent contact.

Comparing the results given here for BMV with the corresponding results for wild cucumber mosaic virus (Anderegg *et al.*, 1961) one finds that the protein shells are quite similar. BMV has a slightly smaller diameter but the thickness of the protein coat, its hydration, and its average density are much the same as for wild cucumber mosaic virus. BMV, however, has considerably less RNA (molecular weight of 1.0×10^6 as compared to 2.4×10^6) and this circumstance leads to two notable differences between BMV and wild cucumber mosaic virus in the structural arrangement of the RNA. In the first place the RNA in BMV does not extend all the way to the center of the virus and secondly the region occupied by the RNA has a greater hydration and lower density than the corresponding region in wild cucumber mosaic virus.

This work was aided by grants from the National Institutes of Health.

Received for publication, November 13, 1962.

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